



Comparative karyotype analysis of populations in the *Alstroemeria presliana* Herbert (Alstroemeriaceae) complex in Chile

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Abstract

Alstroemeria L., one of the most diverse genera of the Chilean flora and of high floricultural value, is represented by 35 species, most of them distributed between 28-38° S in the Mediterranean zone of Central Chile. There are 24 complex-forming taxa, of which 18 have conservation problems (8 are considered “endangered” and 10 as “vulnerable”). One of these complexes is *Alstroemeria presliana* Herb. with two subspecies: subsp. *presliana* and subsp. *australis* Bayer. *Alstroemeria presliana* grows in Chile and Argentina: subsp. *presliana* is distributed from Reserva Nacional Siete Tazas (35°27' S, Region of Maule) to Antuco, (37°25' S, Region of Bío-Bío), and is also found in Neuquén, Argentina; subsp. *australis* is endemic to the Cordillera of Nahuelbuta. A comparative karyotype study was carried out among six populations of *A. presliana* subsp. *presliana* and five populations of *A. presliana* subsp. *australis*. The eleven populations presented an asymmetric karyotype, with $2n = 2x = 16$ chromosomes but with different karyotype formulae. *A. presliana* subsp. *presliana* shows the haploid formula $2m + 2m\text{-sat} + 1sm\text{-sat} + 1st\text{-sat} + 1t + 1t\text{-sat}$, and *A. presliana* subsp. *australis* presents a formula $1m + 2m\text{-sat} + 1sm + 2t + 2t\text{-sat}$ chromosomes. The architecture of the karyotype between the subspecies is very different. The scatter plot among CV_{CL} vs. M_{CA} shows different groupings between populations of the two subspecies. According to the results obtained it is possible to consider raising *Alstroemeria presliana* subsp. *australis* at species level.

Keywords: *Alstroemeria*, asymmetry, Chile, karyotype, species complex.

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Introduction

Chile has important endemic biodiversity, especially in its vascular flora. Forty-six percent of the Chilean vascular taxa are endemic (Marticorena, 1990), and many of them present conservation problems (Muñoz and Moreira, 2003). For this reason, Chile has been included among the world biodiversity hotspots (Arroyo *et al.*, 1999; Myers *et al.*, 2000; Mittermeier *et al.*, 2004). The large environmental diversity of Chile (Cowling *et al.*, 1996; Arroyo *et al.*, 2008) and consequently the different selective pressures, would likely stimulate active micro-evolutionary processes that would result in high floristic diversity, as well as high levels of endemism. Several parameters contribute to this ecological heterogeneity: the wide latitudinal and altitudinal gradient, the insular character, and the historical isolation from the rest of the vegetation of the continent (Villagrán and Hinojoza, 1997; Armesto *et al.*, 1998; Lazo *et al.*, 2008). This would also have implications for the occur-

rence of subspecific and/or varietal complexes that are very difficult to resolve taxonomically.

A zone of special interest in the Chilean hotspot is the Mediterranean zone, which extends between 23° and 38° S (Amigo and Ramirez, 1998), and which harbors a great diversity of environments (Di Castri, 1981). In this region, several plant genera exhibit their maximum floristic diversification and high levels of endemism, such as *Adesmia* (Burkart, 1967), *Alstroemeria* (Bayer, 1987; Muñoz and Moreira, 2003; Moreira, 2011), *Calceolaria* (Ehrhart, 2000), and *Escallonia* (Sleumer, 1968). As in most zones with Mediterranean climate of the world, in central Chile there is a large number of species with restricted distribution and with high probability of extinction (Cowling *et al.*, 1996). Moreover, this is also the zone of the country that concentrates the highest human population, accompanied by intense anthropic intervention due to agriculture and forest activities, as well as urbanization (Vergara *et al.*, 2006).

Economic utilization of the floristic diversity within Chile has led to a vibrant floriculture market that has undergone strong growth in the last decade. One of the families with a large number of endemic species in Chile is Alstroemeriaceae, which contains several species with high po-

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tential for the floriculture market (Baeza *et al.*, 2011 a,b). Given the beauty of the flowers, Chilean species of *Alstroemeria* have achieved worldwide recognition as desirable ornamental and cut plants (Buitendijk and Ramanan, 1996; Buitendijk *et al.*, 1997), and many species have attained a high commercial value (Stephens *et al.*, 1993). Countries such as Holland, England, Japan and the United States have created breeding and propagation programs for commercial species (Miyake *et al.*, 1989; Jeu *et al.*, 1992), and hybrids originated from Chilean species by means of genetic engineering have come back to their place of origin as import products (Muñoz and Moreira, 2003). A good example of this is provided by species such as *A. aurea* R. Graham, *A. ligtu* L. and *A. pelegrina* L., among others. Many cultivars have originated from site-specific breeding that consists of haploid, triploid and tetraploid forms, with increased beauty and duration of the flowers (Kuipers *et al.*, 1997). Morphologically, Chilean species of *Alstroemeria* have been studied in detail, first by Bayer (1987) who described, illustrated, provided distributions, and included photographs of 42 taxa in 31 species. Later, Muñoz (2000, 2003) described 3 new species and one variety, and finally, Muñoz and Moreira (2003) published a beautifully illustrated book on the biodiversity, distribution, and conservation of this genus in Chile.

Alstroemeria is one of the most diverse genera of the Chilean flora, and it is represented by 35 species, most of them distributed between 28° S and 39° S (Muñoz and Moreira, 2003) in the Mediterranean zone (Amigo and Ramírez, 1998). Eleven species of this genus contain complexes of two to four intraspecific taxonomic entities (subspecies and varieties). Representatives in each of these complexes grow in the Chilean Mediterranean zone and some of them are endemic to this region, with a very restricted distribution (Bayer, 1987; Muñoz and Moreira, 2003). *Alstroemeria presliana* Herb. includes two subspecies: subsp. *presliana* (Figure 1A), and subsp. *australis* Bayer (Figure 1B). They grow in a restricted fashion in Chile from the cordillera of Curicó (35°27' S) to the cordillera of Antuco (37°25' S), and from 1500 to 2000 m elevation; they also occur in Neuquén Province of Argentina. *Alstroemeria presliana* subsp. *australis* is endemic to Chile, occupying a narrow geographic distribution, from Curanilahue (37°23' S) south to the river Cautin (38°29' S), and from 200-1500 m elevation (Muñoz and Moreira, 2003). Baeza *et al.* (2008) completed a comparative karyotype study of one population of *A. presliana* subsp. *presliana* and one population of subsp. *australis*, which revealed two different karyotype formulae. It would therefore, be very interesting, to analyze more populations of both subspecies to determine the stability of karyotype structure within each. Both subspecies have attractive pink flowers that can be differentiated primarily by the size and color of their tepals, in addition to geographic distribution. This species represents, therefore, a high potential for development as an or-

namental plant that has so far not been successfully developed.

The study of karyotype is a valuable tool for comparison of populations either within the same or among different species (Schrader *et al.*, 2000, 2003; Matsumoto *et al.*, 2000). The study of karyotype variation is important not only as a tool for evidence of sources of genetic variability, but also because it represents an important micro-morphological feature for understanding evolution within species. Detailed analysis of the chromosomes of related taxa can provide valuable information concerning their evolution and taxonomy (Dimitrova and Greilhuber, 2000). The objective of this research, therefore, has been to cytogenetically characterize and compare the two subspecies of the *A. presliana* complex throughout its entire distribution, and to clarify their taxonomic status.

Materials and Methods

Plant material

The material of *Alstroemeria presliana* analyzed came from 11 populations, six from subsp. *presliana* and five from subsp. *australis* (Figure 1C). Vouchers were deposited in the herbarium of the Universidad de Concepción (CONC). Sources of material were as follows:

Alstroemeria presliana subsp. *presliana*: Región del Bío-Bío, Provincia de Biobío, Comuna de Yungay, Hacienda Rucamanqui, Sendero Cerro Manque, 1200 m, 37°11' S, 71°43' W, C. Baeza & J. Espejo 4362 (CONC). Región del Bío-Bío, Provincia de Ñuble, Comuna de Pinto, entrada a Fundo Los Pellines, 727 m (36°49' S, 71°36' W), C. Baeza 4364 (CONC). Región del Bío-Bío, Provincia de Ñuble, Comuna de Pinto, Fundo Los Pretiles, sector Las Piedras, 1300 m (36°53' S, 71°36' W), C. Baeza 4365 (CONC). Región del Bío-Bío, Provincia de Ñuble, Termas de Chillán, ca. 500 m más arriba del Hotel Termas de Chillán, a 50 m de La Virgen, 1608 m (36°54' S-71°24' W), C. Baeza 4192 (CONC). Región del Bío-Bío, Provincia de Ñuble, Comuna de Pinto, Frente a la entrada a la Cueva de Los Pincheira 1000 m (36°53' S-71°33' W), C. Baeza 4372 (CONC). VII Región, Provincia de Curicó, Comuna de Molina. Parque Nacional Radal 7 Tazas, sendero Malacara, C. Baeza 4373.

Alstroemeria presliana subsp. *australis*. Region of the Araucanía, Provincia de Malleco, camino al Parque Nacional Nahuelbuta, a 500 m de Vegas Blancas, bosquecito de Roble, 795 m (37°49' S-72°54' W), C. Baeza 4369 (CONC). IX Región, Provincia de Malleco, Parque Nacional Nahuelbuta, Piedra del Águila, 1350 m (37°49' S-73°08' W), C. Baeza 4250 C. IX Región, Provincia de Malleco, camino al Parque Nacional Nahuelbuta, a 50 m de la entrada al Parque, en un bosque de Roble, 1250 m (37°49' S-72°57' W), C. Baeza 4370 (CONC). IX Región, Provincia de Malleco, camino desde el Puente El manzano a Angol, 690 m (37°47' S-72°50' W), C. Baeza 4371

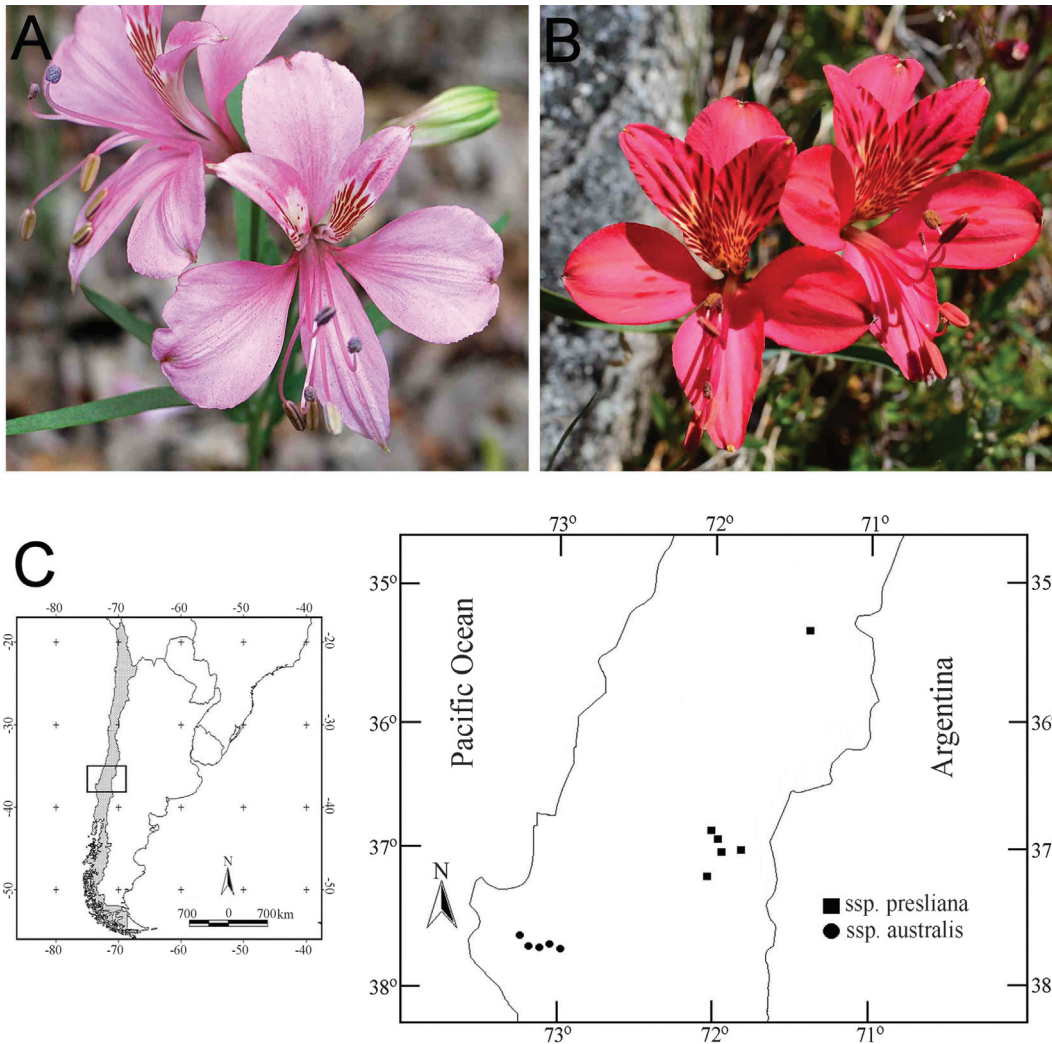


Figure 1 - Photographic representation and geographic distribution of *Alstroemeria presliana*. (A) Photography of *Alstroemeria presliana* subsp. *presliana*; (B) Photography of *Alstroemeria presliana* subsp. *australis*; (C) Geographic distribution of the 11 analyzed populations of *Alstroemeria presliana*.

(CONC). IX Región, Provincia de Malleco, Trongol, Piedra del Queso, Caramávida, 1222 m (37°39' S- 73°06' W), G. Fuentes s.n.

Methodology for the study of karyotypes

Roots (1-2 cm long) obtained from material cultured in a greenhouse were cut and pre-treated with a solution of hydroxyquinoline (2 mM) for 24 h at 4 °C. Subsequently, they were fixed in a mixture of ethanol/acetic acid (3:1) for 24 h. Root tips were then squashed in an acid hydrolysis of HCl 0.5 N for 18 min at 45 °C, followed by washing and staining with 1% orcein. Metaphase plates were photographed with a Zeiss Axioskop microscope equipped with a digital camera, and the pictures were analyzed with Paint Shop Pro Photo X2. Chromosomes were measured with the assistance of the software MicroMeasure 3.3 (Reeves, 2001) and classified according to arm ratios (long arm/short arm; modified from Levan *et al.*, 1964), catego-

rized by position of the centromere: 1.0-1.7 (metacentric, m); 1.7-3.0 (submetacentric, sm); 3.0-7.0 (subtelocentric, st) and 7.0-∞ (telocentric, t). For every population analyzed (10 metaphase plates), the intrachromosomal asymmetry index M_{CA} and the interchromosomal asymmetry index CV_{CL} was calculated (Peruzzi and Eroglu, 2013).

Results and Discussion

The eleven analyzed populations of *Alstroemeria presliana* present $2n = 2x = 16$ chromosomes. *A. presliana* subsp. *presliana* has a haploid formula of $2m + 2m\text{-sat} + 1sm\text{-sat} + 1st\text{-sat} + 1t + 1t\text{-sat}$, *i.e.*, two pairs of metacentric chromosomes, two metacentric chromosomes with satellite, one submetacentric pair with satellite, one subtelocentric pair with satellite, one telocentric pair, and one telocentric pair with satellite. *A. presliana* subsp. *australis* has a haploid formula of $1m + 2m\text{-sat} + 1sm + 2t + 2t\text{-sat}$, *i.e.*, one pair of metacentric chromosomes, two metacentric

pairs with satellite, one submetacentric pair, two telocentric pairs, and two telocentric pairs with satellite. The karyotypes of the two subspecies are shown in Figure 2. Values of the karyotype asymmetry index CV_{CL} and M_{CA} are summarized in Table 1. Figure 3 represents the dispersion of the data in relation to the CV_{CL} and M_{CA} indices of Peruzzi and Eroglu (2013). The analyses are based on ten metaphases from each population.

Preliminary studies (Baeza et al., 2008) in the *Alstroemeria presliana* complex revealed the presence of karyotype variability between subspecies *presliana* and *australis*. This variability was observed both in the karyotype morphology, as well as in the presence or absence of secondary constrictions and satellites. This study, however, included examination of only one population from each subspecies. The present study of five populations of subsp. *australis* and six of subsp. *presliana* have corroborated these differences. In a detailed comparison of the karyotypes (Figure 2), one observes large differences in asymmetry as well as architecture. Subsp. *presliana* has three pairs of large metacentric chromosomes, the second pair with satellites in the terminal zone of the short arm, whereas subsp. *australis* has only two large pairs of metacentric chromosomes. This situation is similar to that reported by Baeza et al. (2010) for the *A. hookeri* complex, where a cytological comparison was made between populations of subsp. *hookeri* of the coast of Region VIII of Chile and of the Pungal de Laja (zone of the intermediate depression). Based on this former study, plus that of Baeza and Ruiz (2011), a new subspecies was described within the complex, *A. hookeri* subsp. *sansebastianana*, endemic to Region VIII. Coincidentally, the populations of *A. hookeri*, as those of *A. presliana* confined to the Nahuelbuta cordillera present karyotypes that are more symmetrical and with large metacentric chromosomes. In both subspecies of *A. presliana*, chromosomes 1 and 2 are identical, the third pair is distinct, chromosome 4 is telocentric in both subspecies,

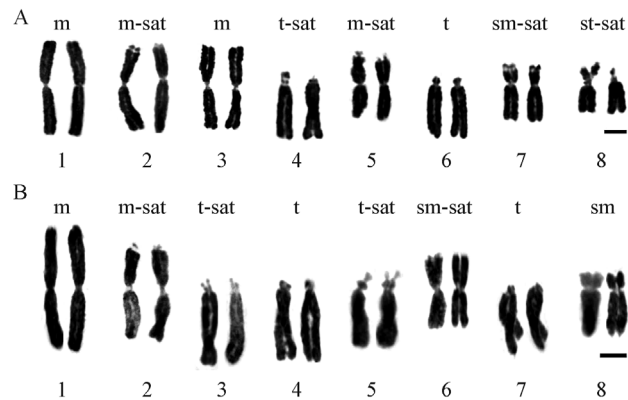


Figure 2 - Karyotypes of the *Alstroemeria presliana* populations (A) *A. presliana* subsp. *presliana* (populations 4192, 4362, 4364, 4365, 4372, and 4373); (B) *A. presliana* subsp. *australis* (populations 4250 C, 4369, 4370, 4371, and G. Fuentes s.n.). Scale bar = 5 μ m.

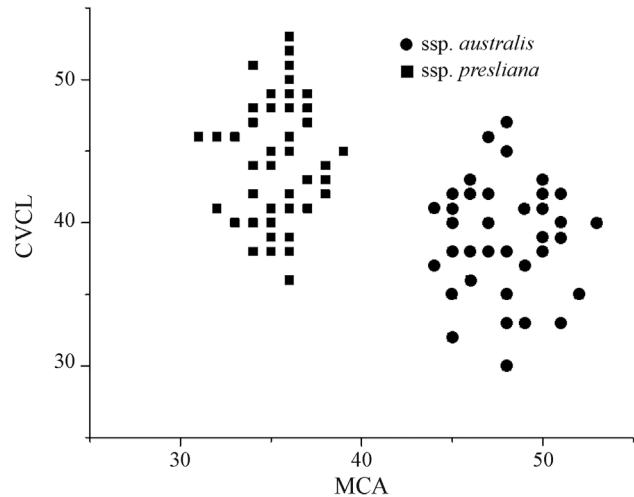


Figure 3 - Scatter plot among populations of *Alstroemeria presliana* subspecies using values of M_{CA} vs. CV_{CL} .

although subsp. *presliana* has a satellite on the short arm. The rest of the chromosomes are dissimilar in karyotype morphology between the two subspecies, which emphasizes their enormous overall difference in architecture. This is further corroborated if one compares the karyotype asymmetry index used (Table 1). Figure 3 shows a diagram of the dispersion of the M_{CA} vs. CV_{CL} values of Peruzzi and Eroglu (2013) based on analyses of all individuals and populations. Two clusters of points can be seen, the squares representing populations of subsp. *presliana* and the circles subsp. *australis*. The major variation is observed in the intrachromosomal index (M_{CA}), as much within each subspecies as between them. Variation is less with the interchromosomal index (CV_{CL}) in the populations of subsp. *presliana* and somewhat greater in subsp. *australis*, although it is very different between the subspecies. Defini-

Table 1 - Karyotype features of the subspecies of *Alstroemeria presliana*. CV_{CL} = Coefficient of variation of chromosome length; M_{CA} = Mean centromeric asymmetric index according to Peruzzi and Eroglu (2013); SD = Standard deviation.

Populations	$CV_{CL} \pm SD$	$M_{CA} \pm SD$
<i>A. presliana</i> subsp. <i>presliana</i> (4192)	39.8 ± 2.70	36.0 ± 0.01
<i>A. presliana</i> subsp. <i>presliana</i> (4362)	44.8 ± 4.96	34.4 ± 0.01
<i>A. presliana</i> subsp. <i>presliana</i> (4364)	45.9 ± 3.48	34.9 ± 0.03
<i>A. presliana</i> subsp. <i>presliana</i> (4365)	42.6 ± 3.92	36.2 ± 0.01
<i>A. presliana</i> subsp. <i>presliana</i> (4372)	46.4 ± 4.25	35.3 ± 0.02
<i>A. presliana</i> subsp. <i>presliana</i> (4373)	44.9 ± 3.97	35.7 ± 0.02
<i>A. presliana</i> subsp. <i>australis</i> (4250 C)	36.3 ± 4.85	48.7 ± 0.01
<i>A. presliana</i> subsp. <i>australis</i> (4369)	42.2 ± 6.12	46.5 ± 0.02
<i>A. presliana</i> subsp. <i>australis</i> (4370)	39.0 ± 4.47	48.8 ± 0.02
<i>A. presliana</i> subsp. <i>australis</i> (4371)	38.4 ± 3.45	49.4 ± 0.02
<i>A. presliana</i> subsp. <i>australis</i> (Fuentes)	38.2 ± 4.39	46.4 ± 0.02

tively, this diagram yields the conclusion that there exists a clear difference in dispersion between the populations of the two subspecies.

Bayer (1987) separated subspecies *presliana* and *australis* based on color of the tepals, length of the external tepals, color and ornamentation of the internal tepals, and anther color. Muñoz and Moreira (2003) considered that the larger, intensely pink flowers are diagnostic features that allow separation of subsp. *australis* from subsp. *presliana*. The cytogenetic results presented here, in combination with a morphometric analysis of tepal color in CIELAB space (unpublished data), provide evidence to suggest species rank for the two taxa. These studies also demonstrate that the subsp. *presliana* grows exclusively in the cordillera of Los Andes and that the subsp. *australis* is endemic to the cordillera of Nahuelbuta. Taken together, we conclude that *A. presliana* subsp. *australis* should be considered as distinct species.

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